Remarks

Reconsideration is respectfully requested in view of the above amendments and following remarks. The specification has been revised to meet the requirements for submitting color drawings. Revisions of claims 1, 16 and 17 are supported, for instance, in the Examples. Revisions of claim 15 are supported, for instance, at page 9, lines 6-21 and in Example 4. Editorial revisions of claims 2, 3, 8 and 18 were made to correct formal matters. No new matter has been added. Claims 1-18 are pending.

Regarding the color drawings in this application, Applicants respectfully submit herewith a petition for color drawings under 37 C.F.R. §1.84(a)(2). Applicants respectfully request acceptance of the color drawings to the extent that the conditions for accepting color drawings have been satisfied.

Claims 2, 3, 8, 15 and 18 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite. Applicants respectfully traverse this rejection, and respectfully request reconsideration in view of the following comments.

As suggested in the Office Action, claims 2, 3, 8 and 18 have been revised to correct grammatically incorrect language. It is respectfully submitted that these claims are definite. Claim 15 recites that the step of subculturing thin slices of the pigmented callus includes adding plant growth regulators to achieve further enhancement of formation of somatic embryos through somatic embryogenesis. Example 4 further supports this language, where the tissue culture is further supplemented with plant growth regulators leading to enhancement of somatic embryogenesis, namely rapid growth and morphogenesis in micropropagules. Accordingly, it is respectfully submitted that claim 15 also is definite.

Favorable consideration and withdrawal of the rejection are respectfully requested.

Claims 1-18 are rejected under 35 U.S.C. 103(a) as being unpatentable over Dawes et al. in view of Mairh et al. Applicants respectfully traverse this rejection, and respectfully request reconsideration in view of the following comments.

Claim 1 is directed to an *in vitro* clonal propagating method for cultivation of marine algae. The invention of claim 1 provides callus induction and plant regeneration via micropropagules that were produced from somatic embryo-like structures, namely pigmented

filamentous callus in an agar culture medium. Further, in order to increase and enhance the production of micropropagules, the invention of claim 1 includes an embedded culture by subculturing thin slices of pigmented callus implanted in the agar of agar plates.

The invention of claim 1 facilitates enhancement in rapid growth and morphogenesis of filamentous callus and led to the finding of development of somatic embryo-like structures in filamentous callus. The present invention provides an advantageous process of algae cultivation wherein a high yielding tissue culture plant clone is used for growing in a bag to produce a biomass similar to that conventionally obtained in open waters without a bag. For example, about a two-fold increase in biomass of tissue cultured plants over control plants can be observed (Example 7). For instance in a 60 day period, the fast growing variant yielded a fresh biomass of 1590 ± 37g (4.6% daily growth rate) compared to that of the control parent plant which only yielded 846 ± 38g (3.6% daily growth rate), and more closely resembled the 1726 g (4.7% growth rate) obtained for conventional plants in open cultivation. Thus, the present invention may provide the advantage of achieving an observed growth rate like that of conventional plants in open cultivation using an enhanced tissue culture while also enjoying the advantages of bag cultivation.

In Dawes et al., tissue culturing is done through propagation of micro-cutting 1-5 mm size explants (p. 250) selected from main axes, particularly where plant regeneration was mainly obtained from mass tissues by culturing 2 mm callus pieces in sterile liquid cultures using plant growth regulators. Mairh et al. is directed to exploring the possibility of bag cultivation of algae on an experimental scale in field conditions.

However, Dawes et al. and Mairh et al., either alone or in combination, fail to teach or suggest the claimed invention, as required by claim 1. Particularly, the cited references do not disclose a tissue culture obtained by subculturing thin slices of pigmented callus as an embedded culture in agar plates thereby further enhancing micropropagule production. Further, the cited references do not teach using the enhanced micropropagule as obtained above to cultivate plantlets in enclosed bags in open sea conditions. Dawes et al. discloses nothing on enhanced micropropagule production in obtaining its tissue culture, namely subculturing thin slices of pigmented callus as an embedded culture in agar plates. Further, Mairh et al. fails to remedy the deficiencies of Dawes et al. In fact, Mairh et al. merely discusses bag cultivation possibilities

and particularly the culture of *Kappaphycus striatum* in a laboratory, in a tank and in the field (tide pools in the intertidal zone) using apical, middle and basal fragments incised obliquely, transversely and longitudinally. Mairh et al. does not disclose subculturing thin slices of pigmented callus as an embedded culture in agar plates and does not disclose cultivation in the open sea. For at least these reasons, Dawes et al. and Mairh et al. do not teach the features of the claim 1.

Furthermore, as the combination of Dawes et al. and Mairh et al. fails to teach the features of claim 1, there is no suggestion that combining the references could lead to any advantages enjoyed by the claimed invention. The present invention provides an advantageous process of algae cultivation wherein a high yielding tissue culture plant clone is used for growing in a bag to produce a biomass as much as that conventionally obtained in open waters without a bag. Even if the teachings of Dawes et al. and Mairh et al. could be combined, which Applicants do not concede, the references fail to derive the features of claim 1 and would not lead to the unexpected results achieved by the claimed invention. Accordingly, it is respectfully submitted that claim 1 and dependent claims therefrom are patentable over Dawes et al. and Mairh et al.

Favorable consideration and withdrawal of the rejection are respectfully requested.

With the above amendments and remarks, Applicants believe that the claims pending in this patent application are in a condition for allowance. Favorable consideration is respectfully requested. If any further questions arise, the Examiner is invited to contact Applicants' representative at the number listed below.

Respectfully Submitted,

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Dated: February 3, 2003

By _____
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Version With Markings Showing Changes Made to Application Serial No. 09/656561

1. (Amended) A [tissue culture] *in vitro* clonal propagating method for cultivation of marine algae, said method comprising the steps of:

a) establishing axenic viable material of [an] algae for tissue culture by sequential treatment of the algal material in sterile sea water supplemented with domestic liquid detergent, povidine iodine and finally incubating the treated material in Provasoli enriched seawater (PES) medium with a broad spectrum antibiotic mixture and a fungicide for about 24 to 96 hours followed by thorough cleaning with sterile sea water to remove any traces of antibiotics and fungicide and blotting with sterile filter paper to obtain axenic explants;

b) culturing the axenic explants on agar plates fortified with PES medium at a temperature ranging between 20-25°C in the presence of cool white fluorescent lights at about 20-50 μ mol photon m^{-2} s^{-1} irradiance and a 12 : 12 light dark cycle for induction of callus;

c) excising the callus from the explant after a period of at least 40 days and subculturing the callus on fresh agar plates fortified with PES medium in the presence of cool white fluorescent lights with 40-60 μ mol photon m^{-2} s^{-1} irradiance and a 12 : 12 light and dark cycle to obtain differentiated densely pigmented oval or spherical shaped micro-propagules;

d) subculturing thin slices of the pigmented callus as an embedded culture in agar plates in Provasoli Enriched Seawater (PES) medium containing plant growth regulators, for a period of about 20 to 40 days, in the presence of cool white fluorescent lights of 20-60 μ mol photon m^{-2} s^{-1} irradiance and a 12 : 12 light and dark cycle to achieve profusely branched pigmented calli in each embedded block leading to enhanced somatic embryogenesis and micro-propagule formation in pigmented filamentous callus;

e) transferring the filamentous calli with somatic embryos to liquid PES medium in an agitated condition for morphogenesis and development of young plantlets with multiple shoots from propagules; and

f) cultivating algal biomass on a large scale in the sea by growing the young plantlets in enclosed perforated polythene bags.

2. (Amended) A method as claimed in claim 1, wherein the material for tissue culture is

a Rhodophytic marine [algae] alga selected from the group of genera of *Eucheuma*, *Gigartina*, and *Chondrus*.

3. (Amended) A method as claimed in claim 1, wherein the material for tissue culture is an [algae] alga selected from the group of *Eucheuma striatum*, *Kappaphycus alvarezii*, *Eucheuma cottonii*, *Eucheuma denticulatum*, *Eucheuma spinosum*, *Eucheuma alvarezii*, *Eucheuma procrusteanum*, *Gigartina intermedia*, *Gigartina exasparata* and *Chondrus crispus*.

8. (Amended) A method as claimed in claim 1, wherein the calli [is] are subcultured by growing thin slices of pigmented calli as embedded cultures in agar plates containing 0.3-0.6% agar and made in provasoli enriched seawater medium at 20-25°C in the presence of cool white fluorescent light at about 20-50 μ mol photon m^{-2} s^{-1} irradiance with 12:12 light and dark cycle to obtain profusely branched filamentous pigmented calli in each embedded block.

15. (Amended) A method as claimed in claim 1, wherein [the process of formation of somatic embryos through somatic embryogenesis of pigmented callus is further enhanced by] said step of subculturing thin slices of the pigmented callus includes adding growth regulators including α-naphthalene acetic acid and 6-benzylaminopurine to achieve further enhancement of formation of somatic embryos through somatic embryogenesis.

16. (Amended) A method as claimed in claim 1, wherein a harvesting period after at least 60 days can yield a higher biomass of tissue cultured plant than that of a control of parent plants or wherein the biomass can be maintained constant and a cultivation period reduced from at least 60 days.

17. (Amended) A method as claimed in claim 1, wherein a two fold increase in growth in fresh weight of tissue cultured plant is achieved over a control of parent plants, without change in carrageenan product yield and gel strength, through micro-propagule formation from pigmented calli.

18. (Amended) A method as claimed in claim 1, wherein the material for tissue culture is a Phaeophytic marine [algae] alga selected from the group of genera of *Luminaria*, *Undaria*, *Ecklonia*, *Eisenia*, *Macrocystis*, *Sargassum*, and *Turbinaria*.